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IFNL3 expression and response to treatment: Behind the HCV tricks

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COMMENTARY ON:

The favorable IFNL3 genotype escapes mRNA decay mediated by AU-rich elements and hepatitis C virus-induced microRNAs. McFarland AP, Horner SM, Jarret A, Joslyn RC, Bindewald E, Shapiro BA, Delker DA, Hagedorn CH, Carrington M, Gale M Jr, Savan R. Nat Immunol. 2014 Jan;15(1):72–9. Copyright © 2014. Reprinted by permission from Macmillan Publishers Ltd. Open access under CC BY-NC-ND license.

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Abstract: *IFNL3, which encodes interferon-λ3 (IFN-λ3), has received considerable attention in the hepatitis C virus (HCV) field, as many independent genome-wide association studies have identified a strong association between polymorphisms near IFNL3 and clearance of HCV. However, the mechanism underlying this association has remained elusive. In this study, we report the identification of a functional polymorphism (rs4803217) in the 3′ untranslated region (UTR) of IFNL3 mRNA that dictated transcript stability. We found that this polymorphism influenced AU-rich element (ARE)-mediated decay (AMD) of IFNL3 mRNA, as well as the binding of HCV-induced microRNAs during infection. Together these pathways mediated robust repression of the unfavorable IFNL3 polymorphism. Our data reveal a previously unknown mechanism by which HCV attenuates the antiviral response and indicate new potential therapeutic targets for HCV treatment.*

While direct acting antivirals for hepatitis C virus (HCV) infection become available in some countries, interferon (IFN)-based regimen remains an option for many patients worldwide [1]. A strong association has been observed between polymorphisms located within the *IFNL3* locus (*IL28B*) and the response to IFN-based therapy [2–4]. Nevertheless, until now no mechanism has been described for the functional effect of these genomic variants.

Several studies have shown an association between favourable and unfavourable polymorphisms and IFNL3 expression in blood and PBMCs from individuals carrying the minor alleles [3,4]. However this association was not demonstrated in infected hepatocytes, stimulated hepatocytes, and infected individuals [5,6], and this association remains controversial. Prokunina *et al.* have shown that a dinucleotide frame shift variant ss469415590, located upstream of *IFNL3*, generates a transcript encoding the IFN-λ4 protein [7]. This is a protein with a 40% amino acidic similarity to INF-λ3. The presence of the dinucleotide variant that allows the expression of IFN-λ4 was associated with a reduced clearance of the virus [7]. Whereas recombinant IFNL4 showed antiviral activity against HCV, it remains a paradox why the presence of IFNL4 is a disadvantage for patients with HCV [8].

In the January 2014 issue of *Nature Immunology*, McFarland *et al.* presented a novel strategy by which HCV reduces the *IFNL3* expression through the induction of two miRNAs that target the 3′UTR region of the unfavourable *IFNL3* variant [9]. The authors identified a genomic variant in the 3′ untranslated region (UTR) of *IFNL3* that determines the *IFNL3* mRNA stability and subsequent IFN-λ3 expression during HCV infection. A thymidine (T) to guanine (G) variation in this region results in a favourable phenotype of sustained virological response (SVR) in HCV-infected patients. This polymorphism, rs4803217 impacts the mRNA decay rate mediated by AU-rich elements and RISC (RNA-induced silencing complex).

The favourable T to G

A-U rich elements (AREs)-mediated decay (AMD) regulates mRNA degradation by the binding of RNA degradative proteins to the mRNA in specific ARE domains. These sites are often present in cytokine mRNAs. McFarland *et al.* described three functional AREs in the 3′UTR region of the *IFNL3*. They observed that the favourable *IFNL3*-G variant presents a higher mRNA half-life than their unfavourable *IFNL3*-T counterpart.

A myosin-linked microRNA regulation

MiRNAs can work together with ARE-binding proteins to induce mRNA degradation [10]. In their study, McFarland *et al.* identified

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two miRNAs (miR-208b and miR-499a-5p) induced by HCV infection, with predicted binding sites at the 3′UTR of the *IFNL3* mRNA. These miRNAs belong to the "myomiRs", a group of miRNAs localised and co-expressed with myosin-encoded genes. Interestingly, the *IFNL3* T-to-G polymorphism localises in the miRNAs seed region and the authors reported that the induced-expression of both miRNAs stimulated the *IFNL3*-T degradation whereas the alternative *IFNL3*-G form was not subjected to this control. Furthermore, HCV infection reduced the expression of the *IFNL3*-T variant but not that of *IFNL3*-G.

miRNA inhibition and viral clearance

The inhibition of miR-208b and miR-499a-5p, in an HCV-infected cell line, rescued the *IFNL3*-T expression with no effect on the *IFNL3*-G variant. Additionally, this increased *IFNL3*-T expression was accompanied with a reduction in HCV copy numbers and virus titer.

The authors complete their observation showing a significant increase in the expression of miR-208b in liver samples of HCV-chronic infected patients. These results emphasise the relevance of the work of McFarland *et al.* presenting viral-induced miRNAs as a mechanism to dampen the host response. Until now, the association between *IFNL3* polymorphisms and its mRNA or protein expression remained controversial. Based on McFarland's demonstration, patients with favourable IFNL3 polymorphism would show increased expression of HCV-induced miRNAs, and reduced protein expression of IFNL3. Further analyses will be needed to determine how the miRNAs, described by McFarland *et al.*, are acting in patients with chronic hepatitis C. A specific regulation of these miRNAs may reconcile the controversial results of the expression level of IFNL3 according to the different genotypes. Moreover, miR-208b, and miR-499a-5p, may regulate the expression of genes encoding antiviral activity and may contribute to the antiviral state associated with the favourable *IFNL3* genotype.

In contrast to previous studies that remained at the genomic level [2–6], McFarland *et al.* went one step further and developed *in vitro* assays to study the effects of these polymorphisms on gene expression. They constructed reporters containing different fluorescent proteins for each of the genomic variants to address simultaneously their expression. This methodology could be applied for the study of other polymorphisms associated to HCV clearance, or interestingly, to address their similar or differential effect in hepatitis B virus (HBV) infection.

Finally, McFarland *et al.* discuss the possibility of developing an anti-miRNA therapy against HCV-induced myomiRs as a promising alternative for patients carrying the *IFNL3*-T variant. However considering the recent advances in anti-HCV therapies, the benefit of the development of anti-miRNAs encoded in myosin genes may be limited. Interestingly, myomiRs expression cannot be induced by any viral infection and seems to be specifically associated with HCV. This finding may explain why IFN polymorphisms have not been associated with IFN-induced viral clearance in other types of viral infection.

In conclusion, McFarland *et al.* provide an elegant study in which they propose a novel molecular mechanism for the previously shown lower IFNL3 expression in patients carrying the unfavourable allele. Together with the description of IFNL4, these studies provide new insights into the molecular mechanisms underlying the association between *IFNL3* polymorphisms and the response to treatment, in patients infected with HCV (Fig. 1). These findings contribute to the understanding of the HCV-host interactions, and the HCV mechanisms for viral evasion. It remains to be established how other polymorphisms in this locus [2–4], are contributing to the response to treatment, and whether direct or indirect mechanisms are involved.

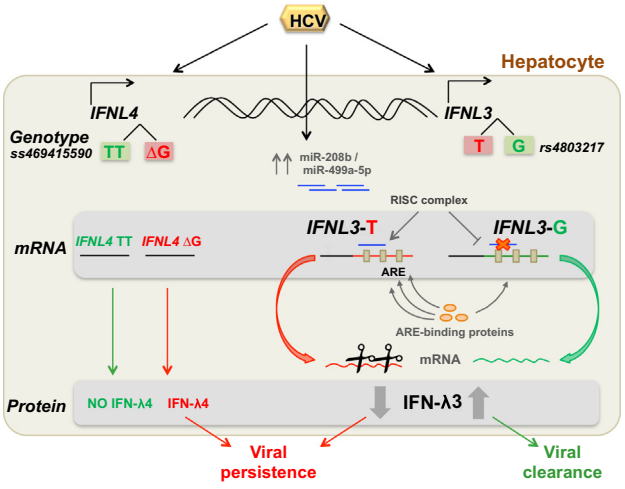

Fig. 1. Schematic representation of molecular mechanisms behind *IFNL3* and *IFNL4* polymorphisms. The polymorphism ss469415590 regulates the expression of IFNL4. The ΔG allele generates a splicing variant that allows the expression of IFNL4 protein, while there is no protein expression with the TT allele. The expression of IFN-λ4 was associated with viral persistence. Regarding the *IFNL3* polymorphism, McFarland *et al.* described that HCV infection induces the expression of two miRNAs (mir-2008b and mir-499a-5p). These two miRNAs target the 3′UTR of *IFNL3* mRNA. In patients carrying the favourable rs4803217 G genotype, these miRNAs cannot bind the *IFNL3* mRNA sequence and therefore cannot inhibit its expression. Conversely, in patients carrying the unfavourable allele, the miRNAs can bind the *IFNL3* mRNA and inhibit its expression by inducing its degradation through the RISC complex. Additionally, the presence of the favourable variant reduces the binding of ARE-binding proteins to the ARE domains, impairing the degradation of the mRNA.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding of conflict of interest with respect to this manuscript.

References

[1] Asselah T, Marcellin P. Second-wave IFN-based triple therapy for HCV genotype 1 infection: simeprevir, faldaprevir, and sofosbuvir. Liver Int 2014;34:60–68. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24373080.

[2] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19684573.

[3] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19749757.

[4] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19749758.

[5] Urban TJ, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. Hepatology 2010;52:1888–1896. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3653303&tool=pmcentrez&rendertype=abstract.

[6] Honda M, Sakai A, Yamashita T, Nakamoto Y, Mizukoshi E, Sakai Y, et al. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. Gastroenterology 2010;139:499–509. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20434452.

[7] Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet 2013;45:164–171. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3793390&tool=pmcentrez&rendertype=abstract.

[8] Hamming OJ, Terczyńska-Dyla E, Vieyres G, Dijkman R, Jørgensen SE, Akhtar H, et al. Interferon lambda 4 signals via the IFN-λ receptor to regulate antiviral activity against HCV and coronaviruses. EMBO J 2013;32:3055–3065. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24169568.

[9] McFarland AP, Horner SM, Jarret A, Joslyn RC, Bindewald E, Shapiro BA, et al. The favorable IFNL3 genotype escapes mRNA decay mediated by AU-rich elements and hepatitis C virus-induced microRNAs. Nat Immunol 2014;15:72–79. http://dx.doi.org/10.1038/ni.2758. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24241692.

[10] O'Neill LA, Sheedy FJ, McCoy CE. MicroRNAs: the fine-tuners of Toll-like receptor signalling. Nat Rev Immunol 2011;11:163–175. Available from: http://www.nature.com/nri/journal/v11/n3/abs/nri2957.html.